Monitoring Reproductive Status by Fecal Progesterone Analysis in Ruminants

Satoko HIRATA and Yuji MORI

Japanese Wildlife Research Center, Bunkyo-ku, Tokyo 113 and Laboratory of Veterinary Ethology, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

(Received 6 December 1994/Accepted 25 May 1995)

ABSTRACT. Characterization of the estrous cycle and the ability to detect pregnancy are essential for the development of efficient captive breeding programs in wild mammalian species and for their conservation. We assess here the feasibility of using fecal progesterone analysis as a non-invasive method of monitoring the ovarian function of the Shiba goat as an experimental model for ruminant species. Feces and blood samples were collected sequentially from 4 female goats throughout the estrous cycle and gestation period and from 2 ovariectomized goats which were subcutaneously implanted with progesterone. The recovery rate with ether extraction was about 70% for fecal progesterone. Fecal progesterone concentrations changed dramatically in accordance with plasma progesterone during the estrous cycle and the pregnancy. Moreover, implantation and removal of progesterone capsules resulted in matched elevation and decline in fecal and plasma progesterone. The present data suggest that measurement of progesterone in feces is a potential noninvasive method of assessing the reproductive status of ruminant species.—key words: feces, goat, progesterone, radioimmunoassay, reproduction


Studies of reproductive physiology have made remarkable progress in domestic ruminants by utilizing radioimmunoassays for monitoring reproductive hormones in the peripheral circulation. In wild ruminant species, however, available information is limited due to the difficulty and plausible stress associated with capturing them for blood sampling, which may affect the animals' physiological condition, including the reproductive function [8, 27, 33]. In this context, development of a noninvasive technique, by which the endocrine milieu of the animals can be assessed even without restricting them, is desirable. The estimation of urinary steroids has been used extensively to monitor the physiological status of animals [20, 32], and urine has even been collected from the ground, squeezed from sand, or cut frozen from snow for this purpose [16]. Frequent urine collection has, however, proved impractical under most conditions. Some trials were also made for analyzing steroids excreted in saliva [10, 12] and milk [10], but obtaining these samples is similarly difficult.

Feces seem most suitable for applying to wildlife, because they are usually much easier to obtain than urine and other samples. Studies of fecal steroid hormones were first conducted mainly on their metabolism and excretion [1–3, 6, 9, 19, 21], but recently, feces have also been used as a source of steroids for the detection of pregnancy or for tracing ovarian cycle in some domestic species and zoo animals [5, 7, 11, 17, 18, 23, 28–30, 34, 36]. However, available information on fecal steroid measurement is still limited and the purpose of the present study was to develop an assay method for fecal progesterone concentration, and to assess the feasibility of it as a practical noninvasive method of monitoring the reproductive function of ruminant species. We used the Shiba goat as an experimental model animal, because a closed colony of these animals has been established for research purposes, and basic information on their reproductive physiology is available [13–15], as a first step to applying this method to wild ungulates such as Japanese deer and Japanese serow.

MATERIALS AND METHODS

Animals: Four fertile female and two ovariectomized Shiba goats were used. The ovariectomized goats were implanted subcutaneously with silicone capsules containing progesterone to mimic the luteal phase progesterone level for 8 days as described elsewhere[24]. They were kept in outside pens and fed daily with hay, concentrates (All-in-one Co.) and pelleted foodstuff (Asemare, Nihon Nosan Kagyo Co.)

Sampling: From females goats, matched samples of feces and blood were collected every other day during the estrous cycle and weekly throughout the gestation period. Matched samples were collected from the ovariectomized animals every day from the day of the subcutaneous implantation of the progesterone capsules [24] through 4 days after their removal. Freshly excreted feces were collected by following a freely behaving animal, and then the animal was caught for blood sampling by jugular venepuncture. All the fecal and blood sampling was performed between 8:00 and 12:00. The feces were kept in a vinyl bag and stored at -20°C within 10 min of collection until assayed for progesterone concentration. Plasma was separated by immediate centrifugation and also kept at -20°C. Some fecal samples were kept at room temperature for 0–48 hr after defecation before being frozen for storage to examine the effect of the pre-freezing time on progesterone concentrations.

Extraction: The extraction procedure for fecal progesterone is summarized in Fig. 1. The fecal samples
were thawed, dried in an oven at 100°C for 1.5 hr, and extracted with diethyl ether. This method was originally developed for use in cattle and caribous [7, 23]. We examined the extraction efficiency of fecal progesterone through varying repetitive numbers, or durations, of extraction and with different volumes of ethanol added for transferring the extract to the assay tube. Based on the information obtained, the following extraction protocol was adopted.

Distilled water (1.5 ml) was added to 0.25 g of dried, crushed feces in an extraction tube with a teflon-sealed cap, and was well mixed before adding 5 ml of diethyl ether. After 10 min extraction with a vortex mixer, the ether layer was collected by snap-freezing and decantation in a tube for drying at 42°C. The wall of the test tube was rinsed with 0.5 ml ether, which was then evaporated under a nitrogen gas stream. The residue was then redissolved with 2 ml of ethanol and vortex-mixed for 10 min. This ethanol solution was diluted 10- to 100-fold with phosphate buffered saline containing 1% bovine serum albumin (PBS-BSA). A 100 μl aliquot of this solution was assayed for progesterone concentration in triplicate. The recovery rate during the extraction of 3H-progesterone added to feces was about 70%. Known amounts of progesterone (20–320 ng) were added to dried feces obtained from ovariectomized goats and assayed for progesterone concentrations. There was a high correlation (r=0.99) between added and recovered progesterone as shown in Fig. 2.

Radioimmunoassay: Plasma and fecal concentrations of progesterone were measured by a double-antibody radioimmunoassay using anti-progesterone rabbit antiserum [22, 26], and 3H-progesterone (Amersham-TRK641). The fecal progesterone concentration was expressed in terms of ng/g of dried feces. The parallelism between the displacement curves for the reference standard of progesterone and the serial dilution of fecal extracts from intact and ovariectomized goats is shown in Fig. 3. The minimum detectable level was 0.085 ng/ml. The intra- and inter-assay coefficients of variation in fecal progesterone were 8.7% and 11.7%, respectively, and those for plasma were 8.7% and 18.8%, respectively.

RESULTS

The fecal progesterone showed a dramatic change during the estrous cycle of female goats as shown in Fig. 4. The pattern of fecal progesterone was virtually identical to that of plasma progesterone with slight delay of a day or two. Plasma progesterone increased from 0.36 ± 0.04 (mean ± SEM, n=4) ng/ml at the follicular phase, up to 8.29 ± 0.56 ng/ml at the mid-luteal phase, whereas fecal progesterone
increased from 41.87 ± 2.16 ng/g to 241.31 ± 17.0 ng/g.

The changes in the fecal progesterone of pregnant goats were also similar to those in the plasma progesterone, and after fertile mating a high fecal progesterone level was maintained throughout the gestation period until it declined abruptly after delivery as shown in Fig. 5. The mean progesterone levels during pregnancy were 8.56 ± 0.59 ng/ml for plasma and 435.59 ± 33.58 ng/g for feces, respectively.

The implantation of progesterone capsules resulted in

Fig. 4. Correspondence between fecal (●) and plasma (○) progesterone levels in intact goats during the ovulatory cycle and early pregnancy.

Fig. 5. Fecal (●) and plasma (○) progesterone levels in two pregnant goats over the gestation period.

increases in both the plasma and fecal progesterone concentrations of the ovariectomized goats as shown in Fig. 6. The plasma progesterone elevated within a few hours of implantation and had declined the day after removal of the implant, whereas fecal progesterone was found to reach a peak on the day after implantation and return to the pre-treatment basal levels a few days after removal of the implant. The mean plasma and fecal progesterone concentrations during the treatment were 5.57 ± 1.78 ng/ml and 84.15 ± 1.78 ng/g, respectively.

The progesterone concentrations in the same fecal samples which were kept at room temperature for 0–48 hr after defecation before freezing for storage are shown in Fig. 7. Even within 30 min of defecation, progesterone concentrations had increased significantly (p<0.05) compared with that of immediately frozen samples, and the tendency continued until the concentration reached a plateau level 24 hr after defecation.

DISCUSSION

The fecal concentrations of progesterone changed dramatically in parallel with plasma progesterone over the

Fig. 6. Fecal (●) and plasma (○) progesterone levels in two ovariectomized goats implanted with subcutaneous capsules containing progesterone. The period of progesterone implantation is shown as the hatched bar at the top.
The plasma progesterone increased and decreased rapidly following the implantation and removal of progesterone capsules, whereas fecal progesterone changed much more slowly with delay of a day or two. A similar time lag was observed in baboons in which changes in fecal progesterone were delayed 2 days compared with those of plasma progesterone [35]. The results suggest that exogenously administered progesterone is excreted in the feces within a few days.

The fecal progesterone concentrations gradually increased when the feces were kept at room temperature for 0–48 hr before freezing for storage. A similar change was reported previously in monkeys [34] and goats [11]. Although the reason for this change is unknown, the phenomenon could be interpreted as a result of conversion from conjugated progesterone in the feces to an unconjugated form caused by intestinal microorganisms. It was reported in pregnant women that the fecal progesterone-metabolite pattern changed markedly following ampicillin administration from 69–79% unconjugated metabolites and 19–26% glucuronides under control conditions, to high steroid sulfate contents of 28–44% due to the reduction of the intestinal flora by ampicillin [21]. It is therefore suggested that fecal samples should be collected and frozen for storage preferably at a fixed time after defecation to obtain accurate data. In the present study all the samples were frozen within 10 min of defecation. However, for the sampling of wild animals or zoo animals it is usually very difficult to sample either immediately after, or at a fixed time from defecation. Holtz [11] suggested that the feces should be kept in an unfrozen state for some time or be exposed to an increased temperature prior to analysis until they reached maximum steroid levels. An alternative method suggested by Wasser et al. [34] is that ethanol should be added to the fecal samples soon after sampling, to suppress the conversion of progesterone to the unconjugated form by intestinal flora, when immediate freezing is difficult.

In conclusion, this study has shown that the measurement of fecal progesterone concentrations is an effective method of monitoring the reproductive state of female goats. However, some problems still remain to be solved before this method is applied to other species, especially to wild or zoo animals, because there are several factors such as seasonal changes in feed, contamination of urine and the way of processing samples which may influence the obtained results. It would also be of practical importance to introduce non-radioisotope assay systems such as the enzyme immunoassay, which can eliminate the limitations associated with the usage of radioisotopes to further promote fecal steroid analysis in ecological studies as well as in wildlife management [4, 5, 17, 31].

ACKNOWLEDGEMENTS. We thank Dr. K. Kubokawa, The University of Tokyo, for her kind advice on the assay procedure and Dr. T. Oki for his technical assistance throughout the study.
REFERENCES


35. Wasser, S. K., Thomas, R. I., Nair, P. P., Monfort, S. L., and...